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Current Literature Review
on 2, 4-D

October, 1980

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for the
Environmental Management Division
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This paper is a review of the scientific literature currently available on 2,4-D. The Montana Department of Agriculture, Environmental Management Division, will maintain this information on file. As data from on-going or future studies are reported, conclusions stated herein may be supported or negated.

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HISTORY OF USE

Early research on plant growth regulators began in 1941 by E. J. Kraus, who was investigating the chemical nature behind plant growth regulators. By 1942, Kraus realized the crop-killing potential of some of these growth regulating substances. The U. S. Army took over much of the research in 1944-45, and the war effort gave a tremendous boost to the herbicide industry. In 1944, it was botanist Fanny-Fern Davis who first put 2,4-D to use killing weeds. She recognized the use of the herbicide as a selective weed killer. Due to the many persons involved with the development and subsequent experimentation of the chemical, arguments concerning patent rights and credidation occurred.

The Vietnam war brought about a significant amount of criticism to the chemical Agent Orange, a 50-50 mixture of n-butyl esters of 2,4-D and 2,4,5-T used to defoliate the dense jungle vegetation to prevent ambushes by the enemy. The criticism led to investigations by the National Cancer Institute in 1964 into the possible birth defects and carcinogenic potential of the herbicides. Although the results from the tests proved positive in susceptible species given massive doses, the report was kept confidential to avoid alarming the public with results from tests involving unrealistic conditions.

Public disclosure of the study brought out the possibility that TCDD, a contaminant of 2,4,5-T, was responsible for the birth defects found. Since 2,4-D did not contain any known

amount of TCDD, its use continued without question. A congressional hearing in April of 1970 on "The Effects of 2,4,5-T on Man and the Environment" was initiated.

Within the past three years, reports of miscarriages and human birth defects began to surface in those parts of the country where herbicides were sprayed in forests, particularly in the northwest region. Although no causal relationships could be established to link the spraying with the miscarriages, the public took the news to heart and a general mistrust of the herbicides began. Last year in Mendocino county, California, voters passed an ordinance forbidding aerial spraying of herbicides. Organizations in other northwest communities are studying the possibility of similar bans.

On April 29, 1980, the EPA responded to the situation with an announcement that more information from the manufacturers would be needed to determine whether 2,4-D is safe for humans and the environment, and that their agency would conduct a review of the information received. During the first week of September, 1980, EPA set deadlines for filing results from human health studies. Studies involving acute oral and dermal toxicity, metabolism, neurotoxicity and teratogenicity are due some time in 1981. Reproduction studies are due in 1982 and cancer studies in late 1983.

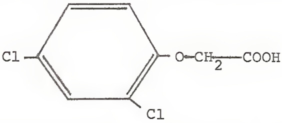
Until the results come in, EPA has placed no ban on any use of 2,4-D. Their present position is that current scientific information does not support the need for regulatory action at the present time.

PHYSICAL AND CHEMICAL PROPERTIES

Synthesis

2,4-D is one of the most commonly used Chlorinated Phenoxy-alkanic Acids. Although available in a variety of forms, most commercial formulations contain an ester or a salt. The free acid is seldom used. Chemical and physical properties of the acid and some commercial formulations can be found in the Herbicide Handbook of the Weed Science Society of America.

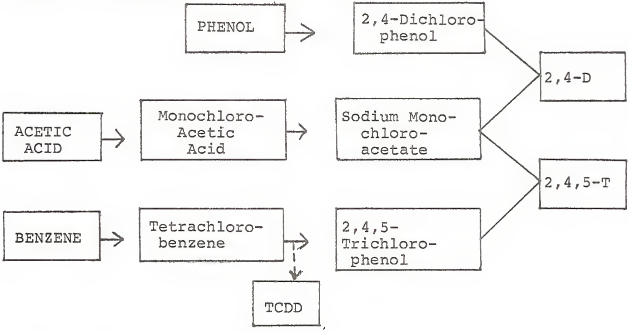
2,4-D



Industrial preparation of 2,4-D involves a two-step process. First step is the chlorination of the phenol, followed by a reaction with chloroacetic acids in the presence of an aqueous solution. The esters are formed by esterification of the acids with the appropriate alcohols. Amines can also be produced from the free acid. The herbicide readily forms salts with alkali metal ions, ammonia and amines (NRCC, 1978). Often times, the public has associated 2,4-D with 2,4,5-T in its production process and the assumption is made that 2,4-D could contain the dioxin TCDD, a major contaminant of 2,4,5-T. There is an important difference in the production processes. 2,4,5-T needs 2,4,5-trichlorophenol as a starting material. When manufacturing this starting material, the toxic impurity, TCDD, may be formed. (See Figure 1). No comparable material

is formed in the 2,4-D process because of the different conditions for the production of the dichloro, and the trichlorophenol (MDH, 1978).

Figure 1 Synthesis of 2,4-D and 2,4,5-T



Formulations

2,4-D is a weak acid that is slightly soluble in water and petroleum oils. The acid is the active form and as stated earlier, is usually converted to water-soluble amines or oil-soluble esters. These formulations are then mixed with other agents depending on the intended use of the herbicides. These include thickeners, emulsifiers, solvents and wetting agents. The strength of the formulation is expressed as pounds of 2,4-D acid equivalents per gallon, or as a percent. Concentrations range from 1 to 80 percent (HALTS, 1980).

Ester formulations generally have greater herbicidal activity than amine forms. Esters are generally more volatile

than salts, although low volatile formulations are manufactured and recommended for use to decrease spray drift. Herbicidal activity will be discussed further in Mode of Action.

Contaminants

There is no report in the literature which cites TCDD contamination in 2,4-D. There have been reports though, concerning other possible contaminants of the compound with other dioxins.

A hexa-dioxin (HCCD) was isolated in a sample of 2,4-D by Woolson et al.(1972). The dioxin first became familiar as a toxic chemical during the late 50's when millions of commercially raised chickens in the United States died after eating commercial poultry food contaminated with HCCD. The source of the HCCD was the chlorophenols isolated in fats added to the feed. An actual tie between this incidence and 2,4-D has not been shown in the literature.

Another dioxin that may be associated with 2,4-D is 2,7-dichlorodibenzo-p-dioxon (DCDD). This is considered to be only slightly toxic. The National Cancer Institute has completed its carcinogenesis bioassay on the compound, but no report has been made as the data was insufficient (NIOSH, 1978). Other chlorinated derivatives of phenoxyacetic have been identified in 2,4-D samples. These are bis (2,4-dichlorophenoxy) methane, bis (2,5-dichlorophenoxy) methane and 2,2,4,6-tetrachlorodiphenoxy methane (HALTS, 1980).

BIOLOGICAL ACTIVITY

Uptake

Phenoxy acids can be absorbed by plant foliage, roots and soft stem tissue (CAST, 1978).

Absorption into the roots by soil application occurs in a few plant species. The mechanism of movement seems to involve absorption by root hairs and cortex parenchyma in the primary region behind the root tip. From here the herbicides move from the symplast where they leak into the apoplast and ascend into the foliage via the transpiration stream of the xylem (Ashton and Crafts, 1973).

Corbin *et al*. (1971) have shown that soil pH has profound effects on the uptake of herbicides by roots. Their experiments conclude that phytotoxicity increased as pH increased and for 2,4-D, it peaked at 6.5 pH. Franadez *et al*. (cited in NRCC, 1978) found the accumulation of 2,4-D by roots increased when the pH was lowered, and some of the 2,4-D could be lost again by the roots to the soil. Once absorbed by the roots, movement is somewhat restricted. The herbicide may be accumulated within the symplast to higher concentrations than the external medium.

More commonly, the herbicide is applied to the foliage. Foliar uptake is essentially a two-step process. The first step is entry into the plant. The second step involves movement through the plant tissues into the living medium. To get into the leaf, the herbicide must cross the waxy cuticle,

which presents an effective barrier against some herbicide formulations. Ammonium and amine salts of the phenoxy acids penetrate the cuticle more readily than the sodium and potassium salts. Lower pH of the application medium allows the free acid to move quickly past the barrier. Esters also readily penetrate the cuticle but short-chain alkyl esters do not translocate as easily as esters made from long-chain alcohols (Loos, 1975).

Adding surface-active compounds as emulsifying agents may increase toxicity by increasing the amount absorbed. Surface-active compounds help dissolve surface waxes and may loosen the structure of the cuticle. Inorganic ions seem to work best as an emulsifier.

An increase in temperatures also promotes increased absorption and subsequent injury to the plant. High relative humidities stimulate the absorption and translocation whereas moisture stress in a plant reduces uptake and movement.

Absorption could also take place through the stomata, although conflicting reports make it difficult to evaluate the importance of this alternate route. Some workers have noted that uptake through the stomata is possible but relatively unimportant. Others claim that 2,4-D application may result in closing of the stomata (Ashton and Crafts, 1973). More field work is needed concerning active absorption through plant stomata.

Application via the stem is also possible with phenoxy herbicides. The process is similar to foliar absorption in

green or succulent stems. Stems covered by bark require an oil carrier when applied as a basal spray. An aqueous solution can be used if a cut is made in the bark first.

For a more detailed explanation of herbicide absorption, please refer to Mode of Action of Herbicides by Ashton and Crafts.

Translocation

Once past the cuticle barrier, the herbicide can move through the plant by either the symplast, including the sieve-tube system of the phloem, or the non-living apoplast, which includes the cell-wall system and the conducting elements of the xylem. Movement with the food supply (phloem) is generally found by researchers.

There is much evidence to support the movement of herbicides from regions of photosynthesis to regions of active growth, where the food supply is needed. So active movement of foods in the plant is a requirement for thorough distribution of the herbicide. In mature plants, the phloem-mobile herbicide applied to the upper foliage moves into the shoot tips, flowers and fruits, while the molecules applied to lower leaves move into the roots. Those mature leaves that are contributing to the food supply are passed by. Transport in seedlings is into the roots. The movement of the herbicide from xylem to phloem and vis versa is possible although not common (NCRR, 1978).

The movement of 2,4-D and 2,4,5-T in the phloem is

restricted compared with other phenoxy herbicides. In the phloem, the less mobile herbicide compounds tend to accumulate in the parenchyma and move out of the mainstream. As a result, the concentration of 2,4-D and 2,4,5-T in the phloem is constantly diminishing, reducing the desired herbicide effect on the plant (Loos, 1975). The more rapid the translocation process, due to increased food movement, the less likely the herbicide will accumulate in the cells. Weed control is best achieved when the plant is undergoing vigorous root growth, with the food moving rapidly down to the growing roots, carrying along with it the herbicide.

An accumulation problem also appears when these herbicides are applied to the roots. They may become bound to living cells in the roots. Root translocation can be a benefit to less phloem-mobile herbicides since movement from roots is via the xylem. When dosage is increased sufficient to produce strong contact action, the herbicides penetrate and move rapidly in the transpiration stream resulting in rapid death of the plant.

Mode of Action

2,4-D belongs to the large group of plant growth-regulators designated as auxins. Auxin is an organic substance which, at low concentrations (less than 10^{-3}M) promotes growth along the longitudinal axis of shoots and inhibit elongation of roots (Loos, 1975). Phenoxy acids are synthetic auxins though, and do not always have the same effects on a plant as natural

auxins do. Normal control of growth is lost in the case of herbicides, as they do not respond to those mechanisms that control natural auxin concentrations. Phenoxy herbicides can also behave as antiauxins, which prevent auxins from reacting in the manner needed to trigger growth response in the plant.

Synthetic auxins stimulate nucleic acid and protein synthesis which leads to a further stimulation of cell growth. This growth can be abnormal. The death of the plant begins with the suppression of normal apical growth along with the initiation of abnormal axis growth. Robertson and Kirkwood (cited in NCRR, 1978) reviewed the research on mode of action and concluded that action of phenoxy herbicides involves a complex series of reactions that begin with the depression of the gene regulating synthesis of the enzyme RNAase. This results in the syntheses of RNA and protein which in turn, brings about "massive" cell proliferation. The proliferation, which usually occurs in the stem, leads to the disruption of the transpiration and translocation systems, resulting in the accumulation of assimilate in the shoots and starvation of the roots. The vascular tissues may become so plugged as to result in lack of nutrients to areas in the plant normally supplied via these tissues. The ability to absorb water and salts is affected by the herbicide as well as inhibition of photosynthesis.

A summary of generalized effects are as follows: in susceptible seedlings normal growth pattern changes rapidly,

meristematic cells cease dividing, and elongating cells stop length growth but continue radial growth. In mature plant parts, parenchyma cells swell and divide, root elongation stops and root tips swell, and young leaves stop expanding and develop excessive vascular tissues.

The herbicides are selective and the noticeable effects on plants will vary with the species treated and the phenoxy herbicide used. The selectivity of the phenoxy herbicide varies in nature. Internally, a plant may have a successful means of detoxifying the compounds, by altering the mode of action process. Difference in structural features of two plants may render one susceptible, the other not. For example, the effectiveness of phenoxy acids on dicotyledonous and not on monocotyledonous plants could be attributed to the phloem structure. Non-effected monocots have their phloem scattered in bundles, surrounded with protective tissue unlike dicots. Also, movement of the herbicide within the plant is more restricted in monocots than dicots (Loos, 1975).

Among dicotyledons, resistance is determined by not only the rate or amount of herbicide uptake and translocation, but also the metabolism and excretion of the herbicide in the plant. Excretion from the roots could be a possible detoxification reaction from some resistant species. Plants which rapidly degrade or bind (conjugate) the phenoxy acid are successful against the actions of the herbicides. Binding the chemical with protein in wheat plants render the herbicide non-toxic (NCRR, 1978). Plants may actively metabolize

the parent compound to nonherbicidal degradation products. Dexter et al.(cited in NCRR, 1978) found that in resistant oats, 2,4-D was immobilized in the leaves and the unaltered, free 2,4-D was reduced to non-toxic concentrations. But in a known susceptible species cocklebur, 2,4-D remained free and mobile. Bromegrass, timothy and orchard grass also convert 2,4-D to a non-lethal compound.

The ability alone of the plant to degrade or conjugate active phenoxy herbicide does not make the plant resistant. The speed of the detoxification process is an important factor.

NITRATE ACCUMULATION

Herbicides have been known to have indirect effects on livestock. Some of these include altering the palatability of the grazed plant, making previously poisonous plants more desirable, or by increasing the nitrate in a plant to toxic levels (Way, 1969).

Some weed species are ordinarily unpalatable to livestock but become palatable after treatment with herbicides. Poisonous plants become an important factor when livestock turn to these species over normally grazed species. The herbicide in this case does not cause an increase in the nitrate level of the plant, but rather increases the use of the plants, which were previously avoided due to unpalatability. These plants may naturally have a high nitrate level and therefore will be toxic to the livestock.

The toxic effects of nitrate are caused by a reduction of nitrate to nitrite and the conversion by nitrite of hemoglobin in the blood to methoglobin. Since methoglobin cannot give up its oxygen to the tissues, the animal dies from asphyxia (Frank and Grigsby, 1957). Abortions in cattle have also been correlated with occurance of high nitrate levels in plants (Sund _et al_., 1960)

Frank and Grigsby (1957) studied this possible connection between herbicides and nitrate accumulation in certain weed species, testing some commonly used herbicides, including 2,4-D in the isopropyl ester form. The weeds were treated

with sublethal dosages. They determined that only one weed species out of the 14 studied accumulated nitrates due to herbicidal treatment. In general, they found 2,4-D to have a varying effect depending on the species tested, indicating that is may be species specific.

Many variables, both internal and external, determine the amount of absorption and the development of nitrogenous nutrients in plants. Factors such as pH and nitrate concentration of the soil, light, temperature, moisture and oxygen supply are important. Also important is the stage of growth of the plant. It is reported that the period of greatest accumulation of nitrate occurs during the stage of growth immediately preceding the flowering stage (Way, 1969). Adverse growing conditions may also cause nitrate accumulation in some plants.

BEHAVIOR IN WATER

2,4-D will breakdown in water either photochemically or under action of aquatic microorganisms. Degradation is dependent on water temperature, amount of light, aquatic fauna present (aerobic or anaerobic), water circulation, depth of body of water and pH of the water. A review on detectable residues indicated that 2,4-D may persist from three to four weeks in some aquatic ecosystems and up to four months in others (NRCC, 1978).

Photochemical

Light provides the energy for a variety of degradative reactions including oxidation, reduction, and hydrolysis (MDH, 1978).

The ester formulations undergo hydrolysis to the acid form and the amine salts disassociate in solution. Most studies therefore deal only with the acid formulation as the esters and salts are readily broken down in water. In the laboratory, the presence of phenols as degradation products was found but the ultraviolet radiation from sunlight would not be expected to decompose 2,4-D to this point (NCRR, 1978). Although much work has been done on photochemical degradation in the laboratory with "simulated sunlight", the photolysis of 2,4-D under natural sunlight conditions is still unclear. The formation of phenols as a degradation product has been noted in laboratory studies but

not proven in natural waters (Loos, 1975). In the natural environment, it is difficult to differ between photochemical and biological degradation, although the presence of light increases the overall degradation process (Warnock and Lewis, 1978).

Biological

As mentioned above, environmental conditions influence the rates of degradation in water. In shallow, warm lakes and streams which are aerobic, 2,4-D is not likely to persist or accumulate. DeMarco *et al.*(1967) found in warm, natural lake waters 2,4-D degraded within 6 days as compared to cold, anaerobic water environments where it persisted for up to 80 days.

It is reported that if 2,4-D was to accumulate in the water, one likely place to find it would be in the sediment. Breakdown of 2,4-D in bottom mud sediment was investigated by Aly and Faust (1964). They applied 2,4-D to samples taken from two lakes. Lake A had been treated one year earlier with 2,4-D and Lake B had no record of previous treatment. Results indicated that 2,4-D disappeared from the sediment of Lake A within 35 days as compared to 65 days in the sediment taken from Lake B. Frank and Comes (1967) applied a 20% granular formulation to lake water and found that it persisted longer in the bottom sediment than in the water. In cold, deep lakes where water circulation is poor, photolysis and volatilization are minimized, and only anaerobic conditions

exist, persistence of 2,4-D in the organic sediments might be expected.

Surface runoff has been cited as a 2,4-D contamination source for water. When herbicides were sprayed in an Oregon forest, immediate concentrations appeared in the water from the spraying operation. But during a nine month period following the spraying, no further indications of 2,4-D persistence were found (Norris, 1967). This may point to the absence of runoff from or leaching through the soil.

The opposite was found in California when the U.S. Forest Service sprayed the Cleveland National Forest with 2,4-D. The herbicide made its way into the San Gabriel River and ended up in the reservoir system of a town 25 miles from the forest. Five years later, 2,4-D was still detected (Warnock and Lewis, 1978). This clearly illustrates the mobility of the herbicide.

Persistence/Residues

A two year monitoring program of pesticides in water was conducted by the U.S. Geological Survey at twenty sites on major streams in the western United States. Out of the 331 water samples, 40 contained 2,4-D in concentrations ranging from 0.03 - 0.035 mg/l (NCRR, 1978). Waldron (1974) found no confirmed residues in either water or sediment samples collected monthly for one year in several river systems in Ohio. Residues have been noted in farm ponds and wells in Ontario (Frank 1970-1974). Norris (1967) found herbicidal

residues in streams located in or adjacent to sprayed areas of forested regions in Oregon. The further downstream and the longer the post-spray time, the less detectable were the residues. Residues in aquatic fauna and bottom sediment have been noted. During 1969, the TVA recorded 2,4-D levels of .35 ppm in the sediment as compared to .042 ppm in the surface water three months after application in the Guntersville Reservoir (Warnock and Lewis, 1978). Plankton has been noted to draw in 2,4-D. In the same TVA study, the plankton was found to have levels at 1.1 ppm. Residues in the plankton and plant pools have been observed to persist for up to 2-6 months (Wojtalik et al., 1971).

The majority of the studies reviewed support residue levels which are low, if any, and which have no effect on the environment. Long-term studies are needed to evaluate the effect, if any, repeated dosage would have on residues.

BEHAVIOR IN SOIL

The disappearance of many herbicides from soils is a first-order reaction. At any given time, the rate of disappearance is proportional to the herbicidal concentration in the soil (Sheets and Harris, 1965).

2,4-D in the soil can affect the soil microorganisms which can in turn influence soil fertility. Subsequent crops may also be affected. Depending on its fate, the herbicide can be leached into waterways creating a potentially dangerous situation to mammals.

It is generally accepted that the degradation of 2,4-D in the soil is primarily due to the action of aerobic soil microorganisms. Because of better aeration and continuous supply of nutrients from the plant roots, the largest numbers of microorganisms can be found in the upper layer of soil. Since 2,4-D is not reported to penetrate in the soil too deeply, the interaction between the herbicide and degrading microorganism is maximal (Torstensson, 1978). Sterilization of the soil greatly reduces the rate of degradation due to removal of soil organisms. Hernandez and Warren (1950) applied 2,4-D to samples in which the soil had been sterilized and also to a group of control soil samples. In the sterilized soil, 2,4-D was still toxic at the end of 12 weeks. In the unsterilized soil (control), 2,4-D was inactivated at the end of four weeks.

The rate of degradation is dependent on several factors. Warm, moist soils with a rich organic content present optimum

conditions for rapid degradation of 2,4-D. Type of soil is also a factor in determining how long the herbicide will remain in the soil, allowing for microbial breakdown. In general, any soil types or conditions that do not promote microbial populations will not degrade 2,4-D efficiently.

Researchers have found that when 2,4-D reached the soil, there appears to be an initial period when degradation is minimal, if any. This is followed by the rapid degradation of the herbicide (Audus, 1960).

The "lag" period in which no action is taking place is characterized as a period of growth and adaptation for the soil microbial population. The microbes are incapable of metabolizing many of the herbicides immediately. The delay before degradation starts is the time required to form the enzymes that catalyze the decomposition of the chemical. The potential of soil organisms to degrade phenoxy herbicides depends on whether or not they possess an enzyme system that normally metabolizes compounds related to the herbicides. If so, the enzyme needed for phenoxy decomposition can be formed from the closely related enzyme already present (Mullison, 1970). Once the readied soil organism begins to increase, degradation begins and accelerates according to the exponential growth of the organism.

In untreated soils, the ability and numbers of organisms with the potential to degrade the phenoxy herbicides are low. But once the soil has been enriched in bacteria able to decompose a particular phenoxyacetic acid, the ability to rapidly degrade the substrate persists for at least one year in the absence of

further degradation (NRCC, 1978). Repeated applications will either shorten or eliminate the lag periods.

The "enriched" bacteria are often capable of degrading other phenoxy acid substrates. Soils that degrade 2,4-D could also detoxicate MCPA. 2,4,5-T could not be degraded in enriched soils previously treated with 2,4-D but could in soils which had earlier been treated with MCPA (Loos, 1975). The rates of degradation of the second molecule will be slower than the rate for the compound which the microbial population was originally developed for. Torstensson (1978) maintains that adaption to a given substrate, for example, 2,4-D, also involves simultaneous adaption to all intermediates in the breakdown chain.

Final products of microbial degradation are humic compounds and/or CO_2 and H_2O and inorganic chlorides.

The question of buildup from year to year is important to growers who are concerned about planting sensitive crops in areas sprayed with herbicides a year or two earlier. According to Sheets and Harris (1965), herbicides that exhibit a lag phase before microbial breakdown are usually not hazardous to subsequent crops. Also, herbicides which injure plants longer than three or four months after application at normal rates can be considered a potential hazard to crops grown in rotation. To be safe, an early application date is advisable. Spraying in the fall increases the chances of residues the next year.

2,4-D is known to leach to some degree in soils. Salts leach more readily than do amines (Wiese and Davis, 1964). Hernandez et al.(1950) notes that the amount of organic matter

in the soil is an important factor in determining the rate that 2,4-D is leached into the soil. In forested areas, leaching would not be considered a significant factor for the possible contamination of ground water because of the high organic content of the forest litter. Helling (cited in NRCC, 1978) classifies 2,4-D as a relatively mobile herbicide.

Persistence/Residues

The persistence of 2,4-D in three soil types was investigated by Altom and Stritzke (1973). The average half-life of 2,4-D was four days while other herbicides tested were considerably longer.

Norris (1966), in laboratory studies, determined the persistence of 2,4-D in forest floor litter. He observed rapid breakdown with almost 90% of the 2,4-D being degraded within two weeks after application.

Repeated applications may reduce the length of persistence of 2,4-D. Torstensson *et al*. (cited in NRCC, 1978) reported soil residues after the first application of 2,4-D at 2.25 kg/ha were toxic to some plant species for ten weeks. A second application one year later resulted in toxic soil residues for only seven weeks. Torstensson *et al*. noted soil which had been treated for 18 consecutive years; the 2,4-D persisted at toxic levels for only four weeks.

Extreme dose levels applied to the soil may overwhelm the microbial population resulting in much longer persistence periods. But, if applied at recommended rates under normal field conditions,

2,4-D would be expected to degrade within a few weeks and would not be carried over to the next season.

RESIDUES IN THE ATMOSPHERE

2,4-D in the air can present potential problems to nearby susceptible crops. Spray drift is recognized as a major cause for the presence of atmospheric residues. Use of low-volatile formulations is recognized as a means of reducing the amount of drift. Research on the modification of the spray equipment as a means to reduce drift is also progressing.

The levels of 2,4-D in the atmosphere are usually determined during the spraying season. Grover *et al.* (1976) examined several air samples from central and southern Saskatchewan during spray season for 2,4-D residues. Up to 50% of the samples collected contained 2,4-D with butyl esters being found most frequently. Samples in one area were collected during or following the two major rainfall periods. The absence of any detectable residue suggests that the herbicide must be washed out of the air periodically. These results were compatible to a similar study carried out in the state of Washington by Adams *et al.* (cited in NRCC, 1978).

In the United States, two separate air monitoring studies near 16 cities across the country found only three samples containing detectable levels of 2,4-D. These cities were Jordan, New York (0.00115 ug/m^3), Rome, New York (0.00154 ug/m^3) and Salt Lake City, Utah (0.004 ug/m^3). The length of the monitoring period was one year (Stanley *et al.*, 1971; Compton *et al.* cited in Grover *et al.*, 1976).

RESIDUES IN ANIMAL TISSUES, MILK AND CREAM

Concern of exposure of livestock to 2,4-D is currently centered on possible residues in the milk, meat by-products and cream. If residues are found, this could be evidence for bioaccumulation of herbicides. Presently, the general concensus is that due to rapid excretion from mammals and degradation in the environment, 2,4-D does not bioaccumulate (HALT, 1980; Gehring and Betso, 1978; Mullison, 1970; MDH, 1978; NRCC, 1978; Leng, 1972).

Leng (1972) conducted feeding studies with 2,4-D and other commonly used herbicides at levels from 10 to 2000 ppm in the total diet of dairy cows, beef cattle, and sheep. Feeding was done in intervals of two to four weeks ending with one week of withdrawal from treated food. After analysis of 1300 milk and cream samples and 1400 meat tissues, only samples given the higher dosage (1000 ppm) indicated some residue. But, after one week of withdrawal of cows from high (1800-2000 ppm) dosage, no residues were detected in most tissues.

Only negligible residues (0.1 ppm) were found in muscle and fat given 2,4-D for four weeks and some residues in the liver and kidneys at doses at or above 1000 ppm. No detectable residues ($<$ 0.05 ppm) remained in most tissues when animals were given untreated feed for one week. So phenoxy herbicide residues decline rapidly in tissues as animals eat untreated food.

Specific tolerances set for milk, meat and meat by-products are listed in Table 1. Leng also states that 2,4-D did not

become concentrated in fatty tissue and that there is no evidence that phenoxy herbicides are found in bound or conjugated form in animal tissues.

A related study by Bjerke et al. (1972) showed similar results for milk and cream residues. Cows were fed a complete ration containing 2,4-D at six levels from 10-1000 ppm for two or three weeks at each level. No residues were found in the milk or cream greater than 0.05 ppm at the 300 ppm or lower feeding levels. At 1000 ppm level, an average residue of 0.06 ppm was found in the milk. Withdrawl from the treated feed resulted in rapid disappearance of residues from milk. At all feeding rates, residues reached a plateau by the second or third day of sampling.

Residues of less than 0.01 ppm of 2,4-D in milk were detected in dairy cows kept out of a pasture one week after it was sprayed at double the normal use rate with ester formulations (Klingman et al., 1966). In the same study, the highest average concentration of 2,4-D residue found in cows allowed to graze in the pasture during the first two days after spraying was 0.03 and 0.02 ppm in the milk.

Clark et al. (1975) also noted a decrease in residue levels shortly after withdrawl of animals from treated food. Cattle and sheep were fed various herbicides for 28 days at four dosage levels (0, 300, 1000, and 2000 ppm). With the exception of the kidneys, 2,4-D residues averaged less than 1 ppm in muscle, fat and liver tissues. They state that because the phenoxy herbicides are excreted unchanged, higher residues in the renal tissues are

to be expected. Again, feeding animals a herbicide free diet for one week reduced the residues present except for in the fat tissue of sheep where an increase in residue was noted. The study concluded that proper agricultural use of herbicides would not result in harmful effects or in more than a minimal residue.

Morton *et al.* (1967) studied the persistence of several popular herbicides, including 2,4-D, on range forage grasses. Rainfall was considered the most important factor influencing residue levels. Rainfall increased the rate of disappearance. It is generally thought that keeping the stock out of sprayed areas for a one to two week period is good prevention against the chance of residues from exposure. Residues on grass immediately after application are not likely to exceed 100 to 150 ppm for each pound of actual herbicide applied per acre (Clark *et al.*, 1975). Half-life is one to two weeks, depending on geographic location.

Chkanikov (1978) studied 2,4-D residues in wheat and feed grains applied at rates of 1 kg/ha and 2 kg/ha respectively. During the first days after treatment, the 2,4-D level was 43.5-56 mg/kg. The residue level halved during the first week after treatment. After three weeks, the level did not exceed 3-4 mg/kg. However, ripe wheat grains contained up to 11 mg/kg. He concludes that 2,4-D concentration decreased in the feed crops due to dilution, the formulation of inactive metabolites and loss of leaves.

The above studies suggest that 2,4-D does not accumulate

in tissues, milk or cream, citing rapid breakdown and subsequent elimination as the reason. But these studies also use language such as "minimal residues", "negligible residues" and "in most tissues", which casts some doubt on the conclusiveness of the study. What is minimal or negligible? The question of metabolite residues has also been brought up, along with the possibility of residue buildup after extended exposure periods. Metabolism of the herbicide will be discussed in a later section. Concerning extended exposure, available data on toxicity of the phenoxy acids suggest that the toxicity, including residue, is mediated by attainment of a given level herbicide, or a steady state. Gehring and Betso (1978) reviewed available toxicity data and concluded that the hazard from repeated, low-level exposure to the phenoxy acids is none.

TABLE 1 - 2,4-D Tolerances for Residues*

The U.S. Government Code of Federal Regulations determined the following tolerances for residues on raw agriculture commodities, food and feed. A complete listing can be obtained from the original document 40CFR 180.142.

Apples, citrus fruits, pears	5.0 ppm
Fruits, stone	0.1N ppm
Potatoes	0.2 ppm
Sugar cane	2.0 ppm
Blueberry, rice	0.1 ppm
Forage of barley, oats, rye and wheat	20.0 ppm
Grain of barley, oats, rye and wheat	0.5 ppm
Corn grain and fresh corn including sweet corn (kernels plus cob with husks removed)	0.5 ppm
Vegetables, leafy (lettuce, cabbage, rhubarb)	0.5 ppm
Vegetables, root crop (beets, carrots, potatoes)	0.1N ppm
Vegetables, seed or pod (peas, beans - all types)	0.1N ppm
Vegetables, fruiting (eggplants, peppers)	0.1N ppm
Melon, pumpkin, squash (winter & summer)	0.1N ppm
Kidney of cattle, goats, hogs, horses and sheep	2.0 ppm
Meat, fat and meat by products (other than kidney) of cattle, goats, hogs, horses and sheep	0.2 ppm
Milk	0.1 ppm
Poultry and eggs	0.05 ppm
Water	0.1 ppm

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* defined as in or on raw agricultural commodities
N negligible residue

ABSORPTION, DISTRIBUTION AND EXCRETION IN MAMMALS

Phenoxy herbicides are absorbed from the gastrointestinal tract and become widely distributed in the tissues (Paulson, 1975). Nothing in the literature reviewed contradicts this. More than 80-85% of an ingested dose is assimilated by mammals under most conditions. The efficiency of the system does not appear to be affected by the magnitude of the dose. Esters may be assimilated less readily than the acids or salts due to decreased absorption from the gut (NRCC, 1978).

Once absorbed from the gastrointestinal tract, the herbicide is reversibly bound to plasma proteins which results in their retention in the plasma and/or extracellular compartments. When a single acute dose is given, an equilibrium is apparently rapidly established between most tissues and the herbicide remaining in the blood (Gehring and Betso, 1978). A consistent pattern of tissue:blood concentration ratio is not always predictable. Ratios may be dose dependent.

The major route of elimination is via the urine, primarily as the parent compound (MDH, 1978). Plasma half-lives of 3-12 hours following a single dose have been reported. Seiler (1978) estimates half-lives to be 10-33 hours and that 75-95% of the substances are excreted within less than 96 hours.

Sauerhoff _et al_. (1977) administered a single dose of 5 mg/kg of 2,4-D to five male volunteers. The average half-life was 17.7 hours. Excretion occurred mainly as 2,4-D (82.3%) with smaller amounts excreted as a conjugate (12.8%).

Due to the rapid elimination of 2,4-D in the urine, an accumulation of phenoxy acids in human tissues in unlikely. There is evidence to support a steady state concentration reached in humans within 3-5 days during repeated administration (Gehring and Betso, 1978; Sauerhoff et al., 1977).

Although urinary excretion of the acid occurs by an active process, large doses may overwhelm this process. Until the system recovers, it may appear as though the herbicide is building up in the body. These residues decline rapidly with a half-life of one to two weeks (Young et al., 1978).

There is concern about the metabolites of phenoxy acids in mammals. It was once believed that the herbicide was excreted unchanged in the urine, but now some metabolic products are being discovered. The Minnesota Department of Health reviewed a study (author not mentioned) where a presumed 2,4-D metabolite, 2,4-dichlorophenol, was identified in the liver and kidneys of sheep.

Erne (cited in Paulson, 1975) reported that pigs excreted 2,4-D in the urine primarily as the parent compound but also as an unidentified acid hydrolyzable conjugate(s). Conjugates can be formed with amino acids such as glycine and taurine and glucuronic acid. The formations of conjugates is dependent on the dose applied. A larger percentage of phenoxy acid is converted into various conjugated forms with higher doses.

In many studies the identity of the compound metabolite or conjugate is not determined due to either the inability to recognize the compound or no attempt to identify it. It is important that all metabolites be identified in case the meta-

bolite proves to be more toxic than the herbicide. Recognizing metabolites will aid in assessing which detrimental effects are produced by the herbicide alone, and which are brought about by metabolic products and conjugates.

Many of the reported excretion studies use labelled 14-C compounds to monitor the excretion process of herbicides in mammals. Data from these experiments may be misinterpreted in the sense that the reported labelled residue may refer to a compound other than the applied herbicide. If 89% of urine excreted was labelled with 14-C, a portion of that 89% may be metabolites or conjugates, but will be interpreted as only the herbicide. This may be a factor in the failure to identify metabolic products of 2,4-D and other herbicides in some studies.

Concern has been voiced for those who work directly with the herbicide, such as forest sprayers, commercial applicators as well as those indirectly exposed to the herbicide from spray drift or residues. In these cases, exposure is mainly to the skin. The degree of skin absorption (percutaneous) of a chemical depends on a number of factors which will all vary with some degree with the individual. The area of the body contaminated is important. Absorption from the scrotum is complete; the head and neck absorb two to six times more than the forearm (NRCC, 1978). Solvents generally increase the absorption powers of the herbicides dissolved in them. Damage to the skin may greatly increase absorption.

ACUTE AND CHRONIC TOXICITY

Toxicity of herbicides can either be acute or chronic. Acute toxicity is the rapid response of organisms to a few large doses of the chemical received over a short period of time. Chronic toxicity is the accumulation of effects resulting from exposure over a long interval (MDH, 1978).

Acute - Phenoxy acids have a low to moderate acute toxicity. Signs of acute toxicity include anorexia, weight loss, depression, muscular weakness, paresthesia, peripheral neuropathy and posterior paralysis with mild liver and kidney effects (Gehring and Betso, 1978). For animals including livestock, the symptoms usually follow a series of events. Locomotory disturbances appear within a few hours. After this comes difficulty in swallowing and general muscle weakness, particularly in the hindquarters which may develop into muscle spasms and inability to coordinate voluntary muscular movements. The animal may or may not fall into a coma. Some animals die suddenly, apparently from ventricular fibrillation (NRCC, 1978; IARC, 1977).

Many of the earlier acute toxicity studies on 2,4-D in the 40's and 50's provided limited information besides LD50 values. The orally administered LD50 values for various species are listed in Table 2. In reviewing acute toxicity of 2,4-D, it is important to remember that the nature of the dose-response relationship varies with both the chemical and the organism. Individauls within a species may also vary, due mainly to age and sex.

In dogs, toxic symptoms were often delayed up to six hours following a single oral, lethal dose. Death appeared to be due, in most cases, to hepatic congestion or pneumonia. Dogs exhibit evidence of liver damage more frequently than other animals (Drill and Hiratzka, cited in Young et al., 1978). However, dogs have a lower capacity to excrete 2,4-D and so it is more toxic to them as compared to other animals.

Acute toxic doses of 2,4-D (765 mg/kg/bw) produce fatty degeneration of the liver, spleen, kidneys and heart in chickens with similar effects in sheep and cattle (IARC, 1977).

For humans, initial symptoms of exposure include irritation of skin, eyes and throat, weakness, headache, nausea, sometimes vomiting and occasionally loss of consciousness. Workers in fields which were previously sprayed with various salt formulations of 2,4-D complained of these symptoms as soon as one hour after starting work. All cases recovered by the next day except for two workers who lost consciousness. They recovered in four days. Even two weeks after a field had been sprayed, farmers working in it still exhibited exposure symptoms (Radionov, 1967).

Accidental exposure comprises the majority of reported cases. Reports range from no effect to death, depending on the dose, formulation, and means of exposure. A farmer who accidently ingested approximately 30 ml of 2,4-D concentrate developed muscle spasms and weakness, generalized skeletal muscle damage and fibrillary twitching. Medical examination ruled out exposure to any other herbicide and the composition

of the ingested compound was known. By calculation, the patient ingested 110 mg/kg/bw of 2,4-D. HALTS (1980) has reviewed four published cases of acute accidental ingestion of 2,4-D only, and reported various effects resulting in death for two of the cases (Nielsen et al., 1965; Dudley and Thapar, 1972; Berwich, 1970; Prescott, 1969).

The LD50 for humans has been estimated between 80 and 800 mg/kg, most frequently at 400 mg/kg. This is based on only a few cases of human exposure and extrapolation from animal data (HALTS, 1980).

Chronic - Workers using various salts and ester of 2,4-D reported symptoms of rapid fatigue, headache, loss of appetite and pains in the regions of the liver and stomach. They also noted a lower sensitivity to smell and taste (Fetisov, cited in IARC, 1977). The original report was lacking numerical data. 220 workers exposed in a manufacturing plant to a daily concentration of 30-400 mg for periods ranging from 0.5-22 years were examined for possible toxic effects. No "meaningful" differences were noted in the exposed group when compared to a control group (Johnson, 1971). This study also lacked supporting data.

Young pigs fed 500 mg of 2,4-D/kg in diet for 2 months developed locomotor disturbances, increasing in severity after one month. Decreased growth rate was reported (IARC, 1977).

No adverse effects were seen in cattle and sheep when fed 50 mg/kg/bw and 100 mg/kg/bw respectively, of a salt and ester formulation of 2,4-D. Doses were fed for 112 days to

the cattle and 481 days to the sheep (Paulson, 1975).

It is difficult to draw a conclusion from chronic feeding studies, as the results depend on formulation factors, length of study period, dose level and experimental animal used. There are some complete reviews on chronic feeding studies in the literature (Young et al., 1978; NRCC, 1978; IARC, 1977; Paulson, 1975; HALTS, 1980).

Many of the studies involving chronic toxicity cases are found in the specific toxicology report such as mutagenicity and carcinogenicity.

TABLE 2 Acute Oral Toxicity of 2,4-D

Herbicide	Formulation	Species	LD 50 (mg/kg/bw)	Reference
2,4-D	Acid	Mouse	368	Rowe & Hymas (1954)
		Mouse	375	Hill & Carlisle (1947)
		Rat	375	Rowe & Hymas (1954)
		Rat	<500	McLaughlin (1951)
		Rat	666	Hill & Carlisle (1947)
		Guinea Pig	<320	McLaughlin (1951)
		Guinea Pig	469	Rowe & Hymas (1954)
		Guinea Pig	1000	Hill & Carlisle (1947)
		Dog	100	Drill & Hiratzka (1953)
		Chicks	541	Rowe & Hymas (1954)
	Butyl ester	Mouse	380*	Konstantinova (1970)
		Mouse	731*	Rowe & Hymas (1954)
		Rat	620*	Rowe & Hymas (1954)
		Rat	920*	Konstantinova (1970)
		Rat	1500*	Schillinger (1960)
		Guinea Pig	848	Rowe & Hymas (1954)
		Cattle	<100	Bjorkland & Erne (1966)
		Chicks	2000	Rowe & Hymas (1954)

TABLE 2 (Continued) Acute Oral Toxicity of 2,4-D

Herbicide	Formulation	Species	LD 50 (mg/kg-bw)	Reference
2,4-D	Sodium Salt	Mouse	360	Loktionov _et al_. (1973)
		Mouse	375	Hill & Carlisle (1947)
				Rowe & Hymas (1954)
		Rat	666	Hill & Carlisle (1947)
		Rat	730	Loktionov _et al_. (1973)
		Rat	805	Rowe & Hymas (1954)
		Rat	2000	Schillinger (1960)
		Guinea Pig	551	Rowe & Hymas (1954)
		Guinea Pig	1000	Hill & Carlisle (1947)
		Chicken	655	Loktionov _et al_. (1973)

* Stupnikov (1972) tested the acute oral toxicity of butyl esters to mice and rats with stated solutions. This may explain the wide spread of results of toxicity within mouse and rat species.

Rat	920-1500 mg/kg-bw	(in water)
	300- 400 mg/kg-bw	(in diesel fuel)
Mouse	380- 640 mg/kg-bw	(in water)

CARCINOGENICITY

The National Cancer Institute has established policy in determining if an agent is carcinogenic. "There must be a significant increase in tumors in a particular tissue of a specific pathological type (i.e. liver carcinoma*) to provide a significant demonstration of carcinogenicity" (HALTS, 1980). Some of the studies done have not met this criteria but continue to be quoted as evidence. A careful look at the results of each study is necessary.

The majority of studies have been done on rats and mice, with a few epidemiological studies done on human populations.

Rats - The Federal Food and Drug Administration conducted a two year feeding study on 25 male and 25 female rats (Osborne-Mendel strain) using five dose levels in the diets (0, 5, 25, 125, 625, or 1,250 mg/kg of diet). The 2,4-D was 96.7% pure and contained no detectable dioxins (sensitivity 1 mg/kg). The total number of rats with malignant tumors were 6 in the control group then 8, 7, 7, 8 and 14 in the treated groups respectively (Hansen et al., 1971). The tumors were not "target organ" types, located in any particular tissue type. Instead, the tumors were randomly distributed types, commonly found in aging rats of this strain. The number of animals surviving the high dosage levels was similar to the control group and lower doses. A significant increase in malignant tumors occurred only in the highest dose in male rats and this was for all sites, not a particular tissue.

In females, there was a significant dose-related trend for tumors, both malignant and benign. The authors concluded that because no single dose level produced a significant increase of a specific type of tumor in a particular tissue, the carcinogenic affect of 2,4-D had not been demonstrated (Hansen et al., 1971).

The study was re-evaluated by Dr. M. Reuber (HALTS, 1980) an independent pathologist. Reuber found that some neoplasms* in the rats may have gone unreported in the original study. His conclusion is that the tumors are of target organ type and that 2,4-D is a carcinogen in rats. The original study and subsequent re-evaluation form the bulk of material on hand concerning cancer in rats.

Mice - In a well known study done by the Bionetics Research Laboratories, mice were given maximum tolerated doses of commercial 2,4-D acid and several esters for up to 18 months orally and subcutaneous. Reporting the results, Innes et al. (1969) found no statistically significant increase in tumor incidence of any type in any sex or strain subgroup. 2,4-D was not carcinogenic in this bioassay, but the experimental design was inadequate. The time span was only 18 months and the number of mice used per group (18) was inadequate for reliable detection of carcinogenesis, even at higher dose levels (HALTS, 1980).

In a study cited by Young et al. (1978), mice given injections of highly purified 2,4-D at rate of 62 mg/kg effectively inhibited development of a particular tumor type

found in this strain of mice.

The International Agency for Research on Cancer (IARC) stated in their 1977 Monograph that all animal studies had limitations either due to inadequate reporting or to the small number of animals used. They concluded "... no evaluation of the carcinogenicity of this compound (2,4-D) could be made".

Humans - It is difficult to isolate 2,4-D exposure only in epidemiological studies because people are exposed to a number of chemicals in their lifetime, some of which may also be toxic.

In 1974, Axelson and Sundell reported that among Swedish railroad workers exposed to different herbicides, a significant, two-fold excess of all cancers was observed in the workers as conpared to the national average. The specific role of 2,4-D was uncertain in this instance because workers were also exposed to 2,4,5-T and amitrole. At first, the excess cancer was attributed to the amitrole and that exposure to the phenoxy acids showed normal tumor incidence (5 cancers at all sites observed versus 2.8 expected). The authors re-examined the data in 1977 and sorted out the effects of amitrole exposure. They found a significant ($P<0.05$) trend indicating a clear relationship with increasing exposure to phenoxy acids. The IARC concluded that the results of this study were not enough to evaluate the carcinogenicity of 2,4-D since it was used with 2,4,5-T which is contaminated with a known toxic dioxin.

Hardell and Sanstrom (1979) found an increased risk for soft tissue sarcomas* related to phenoxy acids (2,4-D, 2,4,5-T) and chlorophenols. It is not possible to show a specific

effect for 2,4-D since many of the exposed individuals were also exposed to 2,4,5-T. Lung cancer in pesticide exposed agricultural workers in Germany was 20 times higher than the expected incidence for specific age groups, but again, exposure was to many different pesticides.

Seiler (1978) cites a study in Sweden involving mesenchymal tumors in persons with a known history of massive exposure to phenoxy acids over periods of 10-20 years. On the national average, more women in Sweden were diagnosed with mesenchymal tumors than men. But in the study area, the male patients outnumbered the females nearly two to one.

Dogs - One other animal study is mentioned in the literature concerning cancer in lab animals. A two-year feeding study done by Hansen et al. (1971) did not produce any significant neoplasms in dogs given doses of 10, 50, 100, and 500 ppm. In reviewing the study, HALTS (1980) claimed there were design flaws in the experiment including too few animals used per dose group and short exposure time of only two years. They state that in cancer bioassays, the dose should be administered over at least 3/4 of the test animals life, which would be 6-9 years in dogs.

There is no conclusive data which states that 2,4-D is a carcinogen. At this point in time, it is only suspected as being carcinogenic. Most authors agree that continued caution with use is the best course of action until further studies can be done with laboratory animals and more information from epidemiology studies comes available.

* carcinoma - malignant tumor which arises in epithelial tissue or lining and covering tissue.

* neoplasms - a new growth of tissue serving no physiologic function; tumor.

* sarcoma - a malignant tumor which affects non-epithelial tissue such as connective tissue, lymphoid tissue, cartilage and bone.

MUTAGENICITY

A mutagen is defined as any inherited alteration in the genetic material. For a substance to act directly as a mutagen, it must interact with DNA, directly or indirectly by affecting a component in the DNA system. The mutations create a diversity of effects. Dominant mutant genes can show up as fetal death, sterility, abnormal numbers of fingers and toes. Recessive mutations may not be expressed for several generations. 2,4-D has been tested for mutagenicity in at least 11 different assays including those using bacterial, yeast, plant and mammalian systems.

Mutagenic activity in microorganisms would confirm the phenoxy acid interaction with DNA synthesis. The bacterial tests have all been negative up to this point. Many of the experiments were spot tests and so they may not have been as sensitive as other assay methods and could not therefore detect a weak genetic activity (Seiler, 1978).

Yeast tests have given both negative and positive results. 2,4-D induced respiration deficiency which can be considered a small or "petite" mutation. Other experiments yielded no effects. The contradiction may have been solved when it was discovered that yeast mutagenicity by phenoxy acid was dependent on the pH that the yeast cultures were treated at. Since 2,4-D is a weak acid, at a lower pH a greater percentage of the compound is in the undissociated form and may more easily enter the cells. This may explain why bacterial tests were all

negative as most experiments done with bacteria were done at a neutral pH (pH 7) and so not much of the 2,4-D may have been able to penetrate into the cell (Zètterberg, 1978).

In higher organisms, assay results were also positive. For Drosophila melanogaster (fruit flies), sex-linked recessive lethality is known to be the most sensitive technique in mutation detection. In this system, 2,4-D had a significant but weak mutagenic activity (HALTS, 1980). The dominant lethal mutations are negative probably due to lack of sensitivity.

To detect mutagenicity in mammalian systems, the testing looks for chromosomal breakage which can be found in various forms. Human lymphocyte cell cultures were studied after applying 2,4-D to the culture. Negative as well as positive results were found. The positive results showed more chromatid-type aberrations than chromosomal aberrations by a ratio of 4:1 (Pilinskaya, as cited in Seiler, 1978).

Yoder et al. (1973) carried out an epidemiologic study on a group of workers highly exposed to 2,4-D, 2,4,5-T, amitrole and atrazine. They were divided into a control group and an exposed group. Testing continued for one year. Chromatid breakage was greatly enhanced in the exposed group during spraying season. During the off season, the exposed group had fewer chromatid gaps and breaks. The probability of 2,4-D being an inducer of chromosomal aberrations is shown. It must be kept in mind though that other chemicals were also used.

With mice, most investigations report positive findings

concerning chromosome breakage under the influence of 2,4-D. No effects were observed in rats. Young *et al.* (1978), after reviewing the literature on the mutation potentials of 2,4-D in animals concluded that 2,4-D did not increase mutation rates nor stimulate a mutagenic response in rats or mice.

The preliminary results from an experiment conducted by Korte and Jalal (1979) with the Norway rat and the house mouse show breaks and gaps in the chromosomes along with varying levels of coiling and banding. Partially banded chromosomes were particularly frequent at lower concentrations of 2,4-D. The authors also believe that the facts are leading to 2,4-D as a potential mutagen to human lymphocyte cells *in culture* at the 50 ug/ml level or higher.

Seiler (1978) states that phenoxy acids do influence nucleic acids and their synthesis, and goes on to say that more information is needed on the penetration of phenoxy acids into cells of different types. Until then, the acids can be considered as potential mutagens but this evaluation is only tentative.

It is important to generate more information on the possible mutagenic effects of 2,4-D because if something is considered mutagenic, it is also suspected of being carcinogenic.

NEUROTOXICITY

Peripheral neuropathy has been reported to occur in cases of acute and chronic exposure incidents. Usually the circumstances involve dermal exposure to an herbicide. The following will be directed toward 2,4-D exposure. In most cases there was no attempt to wash off the herbicide. Only a few cases have been reported while other potential cases may go undiagnosed.

Early symptoms vary with the individual. In some cases headache, nausea, vomiting and abdominal cramps were reported within one to two days after exposure. In other cases initial symptoms were absent. One to two weeks following the initial exposure, numbness and aching of the toes and fingers began, followed by increasing motor and sensory deficits in all extremities, usually more pronounced in the lower and distal extremities. Temporary paralysis was also noted in some cases but paralysis could last up to two years. Recovery from these exposures was generally slow, and sometimes incomplete.

In their report on the human health hazards of 2,4-D, the California Department of Health Services (HALTS, 1980) describes the mechanism of peripheral neuropathy by the destruction of peripheral nerve axons. The loss of nerve axons begins distally and then ascends. Recovery is the regrowth of these axons. Nerve biopsy reveals destruction of individual fibers and conduction times for the nerves are normal or slightly below normal.

The central nervous system can also be affected. In the case of a 23 year old who committed suicide by ingesting 2,4-D, severe degenerative changes of the ganglion cells were found in the central nervous system (Nielsen et al., 1965). This was not cited as cause of death.

2,4-D exposure has been linked to most reported cases of peripheral neuropathy following herbicide exposure. A farmer who repeatedly used his bare hands to unplug his weed sprayer complained of symptoms of stiffness of hands and knees along with numbness of extremities as noted above. The diagnosis was primarily sensory, peripheral polyneuropathy, probably produced by a 40% solution of 2,4-D (MDH, 1978).

In some cases of reported neuropathy, 2,4-D was used in combination with another compound. Forestry workers spraying a mixture of 2,4-D, picloram and ethylene glycol (Tordon 101-^{R}) were daily ,dermally exposed to the herbicide. One worker sprayed himself in the face and neck and became ill with general symptoms of herbicide poisoning. Within one week he developed a severe neuropathy (HALTS, 1980). The worker was the only one affected by the spraying. This may have been due to his increased direct exposure when he sprayed himself, or a greater susceptibility to the herbicide for some unknown reason.

Goldstein et al. (cited in NRCC, 1978) described three cases of 2,4-D ester dermal exposure and concluded that the 2,4-D caused the peripheral nervous disorders found. In one instance, paralysis persisted for several years. Motor and

sensory nerves were affected. No attempt was made to describe the manufacturing or purity of the ester solution so a cause-effect relationship cannot be made.

Those who work with the herbicide are typically exposed by absorption through the skin. The amount absorbed may vary with the individual depending on their susceptibility, the chemical formulation, exposure to other parts of the body and increased exposure due to damage to the skin.

No dosage level has been determined for the initiation of neurotoxic symptoms. Many claims have been made against 2,4-D and its ability to produce a variety of neurotoxic symptoms. In reviewing the literature, it is important to get all the facts surrounding the exposure incident including formulation and type of exposure and whether or not a medical examination was done on the subject. Some people have claimed numbness of legs and hands after kneeling in a garden wet with 2,4-D. No evidence of any type of examination could back up the claim in the report. Most authors advocate more research in the area of dose-related responses in humans regarding neurotoxicity and agree that the information available is incomplete. Nevertheless, the possibility of 2,4-D inducing neurotoxic symptoms does exist.

TERATOGENICITY/EMBRYOTOXICITY

The Minnesota Department of Health defines teratogenic as a developmental disturbance in the embryo, resulting in congenital malformations. They define embryotoxic as any harmful effect on the embryo or fetus. EPA states that feto or embryotoxicity includes teratogenicity (U.S. EPA, 1980). Common examples of fetotoxicity are reduced fetal weight and prenatal survival, abnormal organ weight, delayed growth and maturation, and increased fetal abortions, resorptions and stillbirths. Teratogenicity includes grossly observable birth defects such as cleft palate, club foot, and exemcephaly; also internal defects such as extra ribs or changes in kidney structure. The use of these two terms is not always consistent and there exists an annoying problem of semantics. What Dow Chemical Company considers embryotoxic, other groups consider teratogenic, or there may be no discrimination between the two terms at all! Interpreting studies and reviews on teratogenicity and fetotoxicity need to be done carefully, keeping in mind possible term discrepencies. Generally the studies describe possible adverse effects on the developing fetus. A reference to the abnormality found and what the investigator labels it will be made in the following studies.

Rats - The most commonly cited study in the literature is that of Schwetz et al. (1971). The study included oral administration of the acid formulation of 2,4-D to pregnant rats at levels up to 87.5 mg/kg-bw/day on days 6-15 of gestation. The

results were fetal anomalies such as wavey ribs, decreased fetal body weight, delayed ossification of bone and edema. The author interpreted these as fetotoxic manifestations. Teratogenic effects were not observed at any dose level. 2,4-D did not effect gestation, viability, or lactation. This study established a no-effect level of 25 mg/kg, but other studies have indicated levels up to 50 mg/kg as having no-effect.

Single oral doses of 100-150 mg/kg/day of 2,4-D were given to laboratory rats on days 6-15 of gestation. Skeletal anomalies and tissue damage were noted especially at the higher doses (Khera and McKinley, 1972). The anomalies in this study, which were labelled teratogenic, were referred to as fetotoxic in Schwetz _et al_.

If the dose level given was not enough to affect fertility or litter size, the pups were usually extremely weak, reduced in size and weight at birth and commonly did not survive to weaning.

Mice - The problem of strain related effects or formulation related effects add to the variation of results in most test animals. At the Bionetics Research Laboratory (Innes _et al_., 1969), subcutaneous doses of 2,4-D, including the esters, were given to several strains of mice at levels between 24 and 106 mg/kg-bw/day. Doses were given on days 6-15 of gestation. Teratogenic responses were evident such as cleft palate, small or no eyes, club foot and abnormal kidney formation. Of the several strains of mice used, no single strain was affected by all the test compounds and no single formulation caused a

positive response in all strains. So, the reported results were very strain-specific.

Courtney (1977) established a specific teratogenic response (cleft palates) for both the ester and the acid forms of 2,4-D. Assuming a LD50 of 400 mg/kg for mice, the doses given represented about 1/4 - 1/2 the LD50.

The no-effect level (NOEL) is apparently very high and above the level of any potential environmental exposure. Levels at which teratogenic effects are thought to begin to appear in mammals are at least one hundred fold above the allowable daily intake, cited by the U.S. Health, Education and Welfare Department (MDH, 1978). In general, the minimal doses required to adversely affect offspring in rodent species fall between 1/8 and 1/2 of LD50. Teratogenic effects are observed with doses close to those which cause maternal toxicity. 2,4-D is a "weak teratogen" compared with other phenoxy herbicides (2,4,5-T) in rodent species (HALT, 1980).

Humans - Because 2,4-D is usually associated with 2,4,5-T and its dioxin which is known to cause birth defects, many people believe that 2,4-D can initiate toxic effects during human fetal development. At this time, no information on related human birth defects and 2,4-D are available.

TOXICITY TO BEES

Herbicide toxicity to bees involves more than direct exposure of the bees to the herbicides. The effects of the herbicides on nectar-producing plants and the contamination of their drinking water also figure into the problem.

When bees were directly dusted with 2,4-D, no adverse effects were noted. Nowever, after aerial spraying a field containing nectar-producing plants, some mortality did occur. These results lead Palmer-Jones (1964) to conclude that poisoning of the bees was through the uptake of nectar. It was not clear if the mortality was due to the direct poisoning of the nectar by the herbicide, or whether abnormal plant growth following the spraying altered the nectar chemistry, making it toxic to the bees. King (1969) found that radioactive 2,4-D can be translocated to the nectar of *Poinsettia* and red clover plants, with detection still possible after two to three days.

Bee colonies sprayed with phenoxy herbicides using oil as a carrier caused toxic effects and mortality. Those herbicide formulations using water as a carrier had non-toxic effects, suggesting that the oil, as a carrier in phenoxy herbicide formulations, may be more toxic to the bees than the herbicide itself is (Moffett *et al.*, 1975). They concluded that 2,4-D and 2,4,5-T are relatively non-toxic to honey bees. A feeding study by King (1969) using 2,4-D in a sugar water solution resulted in no apparent detrimental effects to the bees.

Loss of bees can also occur indirectly through reduced

brood rearing. Moffett _et al._ (1975) cite a feeding study relating dosage levels to the number of young raised. Brood rearing was reduced at feeding levels of 100 ppm and completely ceased at 500 ppm. When feeding of the phenoxys stopped, brood rearing returned to normal. Inclusion of 2,4-D to the bees' water supplies brought about the same effects. If another source of water is available nearby which is not contaminated with herbicides, the bees will ignore phenoxy-contaminated water and drink only from the other source.

Residues of 2,4,5-T were found in honey, bees and wax in small amounts for more than one year after discontinued use by the bees of contaminated water. The concentration of 2,4,5-T in this water was 1000 ppm, a level far above the 10 ppm expected under normal circumstances (Morton _et al._, as cited in Moffett, 1975).

LITERATURE CITED

Allenbone, J.E., R.J. Hamilton and B. Ravenscroft. 1975. Environmental organic chemistry of 2,4-dichlorophenoxyacetic. Environmental Chemistry 1:160-190.

Altom, J.S. and J.F. Stritzke. 1973. Degradation of dicambia, piclorm, and four phenoxy herbicides in soils. Weed Science 21:556-560.

Aly, O.M. and S.D. Faust. 1964. Studies on the fate of 2,4-D and ester derivatives in natural surface waters. J. Agric. Food Chem. 12:541-546.

Ashton, F.M. and A.S. Crafts. 1973. Mode of Action of Herbicides. Chapter 6 - Phenoxys. John Wiley and Sons, New York. p. 256-266.

Audus, L.J. 1960. In Herbicides and the Soil. E.K. Woodford and G.R. Sagar, eds. Blackwell, Oxford. p. 1-19.

Axelson, O. and L. Sundell. 1974. Herbicide exposure, mortality and tumor incidence, an epidemiological survey of Swedish railroad workers. Work, Environment and Health 11:21-28.

Axelson, O. and L. Sundell. 1977. Phenoxyacids and cancer. Lakartidningen LXXIV:2887-2888.

Berwick, P. 1970. 2,4-dichlorophenoxyacetic acid poisoning in man. J. Am. Med. Assoc. 214:1114-1117.

Bjerke, E.L., J.L. Hermon, P.W. Miller and J.H. Wetters. 1972. Residue study of phenoxy herbicides in milk and cream. J. Agric. Food Chem. 20:963-967.

Bjorklund, N.E. and K. Erne. 1966. Toxicological studies of phenoxyacetic herbicides in aminals. Acta. Vet. Scand. 7:364-390.

Chkanikov, D.I. 1978. 2,4-D residues in wheat and feed grains. In Russian, from English abstract in Pesticide Abstracts (78-2552) p. 661.

Clark, D.E., J.S. Palmer, R.D. Radeleff, H.R. Crookshank and F.M. Farr. 1975. Residues of chlorophenoxy acid herbicides and their pehnolic metabolites in tissues of sheep and cattle. J. of Agr. Food Chem. 23:573-578.

Compton, B., P.P. Bazydio and G. Zweig. 1972. Field evaluation of methods of collection and analysis of airborne pesticides. II. Pesticide field monitoring data. EPA Contract No. 70-145, NTIS 216 pp.

Corbin, R.T., R.P. Upchurch and F.L. Selman. 1971. Influence of pH on the phytotoxicity of herbicides in soil. Weed Science 19:233-239.

Council For Agricultural Science and Technology (CAST). 1978. The Phenoxy Herbicides. Report No. 77.

Courtney, K.D. 1977. Prenatal effects of herbicides - evaluation by prenatal development index. Archives of Environmental Con-tamination and Toxicology 7(1):3346.

De Marco, J., J.M. Symons and G.G. Robeck. 1967. Behavior of synthetic organixs in stratified impoundments. J. Am. Water Works Assoc. 59:965-976.

Dexter, A.G., F.W. Slife and H.S. Butler. 1971. Detoxification of 2,4-D by several plant species. Weed Science 19:721-726.

Drill, V.A. and T. Hiratzka. 1953. Toxicity of 2,4-dichlorophenoxyacetic acid and 2,4,5-trichlorophenoxyacetic acid. Arch. Ind. Hyg. 7:61-67.

Dudley, A.W. and N.T. Thapar. 1972. Fatal human ingestion of 2,4-D, a common herbicide. Archives of Pathology 94:270-275.

Erne, K. 1966. Distribution and elimination of chlorinated phenoxyacetic acids in animals. Acta. Vet. Scand. 7:240-256.

Fernandex, O.A., M.E. Ordonez, N. Curvetto, L.M. Fernandez and M. Rigato. 1972. Absorcion del acido 2,4-dichlorofenoxiacetico por Spirodela intermedia. W. Koch. Phyton (Buenos Aires) 20:105-117.

Festisov, M.I. 1966. Problems of occupational hygiene in work with herbicides of 2,4-D group. Gig. I. Sanit. 31:28-30.

Frank, P.A. and R.D. Comes. 1967. Herbicidal residues in pond water and hydrosoil. Weeds 15:210-213.

Frank P.A. and B.H. Grigsby. 1957. Effects of herbicidal sprays on nitrate accumulation in certain weed species. Weeds 5:206-217.

Frank, R. 1970-1974. Annual report - analysis of samples connected with herbicide damage investigations. Provincial Pesticide Residue Testing Laboratory, Ontario Ministry of Agriculture and Food, Guelph, Ontario.

Gehring, P.J. and J.E. Betso. 1978. Phenoxy acids: effects and fate in mammals. In: Chlorinated phenoxy acids and their dioxins. Ecological Bulletin 27:122-133.

Goldstein, N.P., P.H. Jones and J.R. Brown. 1959. Peripheral neuropathy after exposure to an ester of dichlorophenoxy acetic acid. J. Am. Med. Assoc. 171:1306-1309.

Grover, R., L.A. Kerr, K. Wallace, K. Yoshida and J. Maybank. 1976. Residues of 2,4-D in air samples from Saskatchewan, 1966-1975. J. Environ. Sci. Health B (11) 4:331-347.

HALTS. 1980. 2,4-dichlorophenoxyacetic acid (2,4-D). Evaluation of the human health hazards. State of California Department of Health Services and Department of Industrial Relations. 40 p.

Hansen, W.H., M.D. Quaife, R.T. Habermann and O.G. Fitzhugh. 1971. Chronic toxicity of 2,4-dichlorophenoxyacetic acid in rats and dogs. Toxicol. Appl. Pharmacol. 20:122-129.

Hardell, L. and A. Sandstrom. 1979. Case control study: Soft tissue sarcomas and exposure to phenoxyacetic acids or chlorophenols. British J. of Cancer vol. 39:711-717.

Hayes, W.J. 1975. Toxicology of Pesticides. The Williams and Wilkins Company, Baltimore, M.D. 580 p.

Helling, C.S. 1971. Pesticide mobility in soils. II. Applications of soil thin-layer chromatography. Proc. Soil Sci. Soc. Am. 35:737-743.

Hernandez, T.P. and G.F. Warren. 1950. Some factors affecting the rate of inactivation and leaching of 2,4-D in different soils. Proc. Am. Soc. Hortic. Sci. 56:287-293.

Hill, E.V. and H. Carlisle. 1947. Toxicity of 2,4-dichlorophenoxyacetic acid for experimental animals. J. Ind. Hyg. Toxicol. 29:85-95.

Innes, J.R.M., B.M. Ulland, M.G. Valerio, L. Petrucelli, L. Fishbein, E.R. Hart, A.J. Pallotta,R.R. Bates, H.L. Falk, J.J. Gart, M. Klein, I. Mitchell and J. Peters. 1969. Bioassay of pesticides and industrial chemicals for tumorigenicity in mice: a preliminary note. J. Natl. Cancer Inst. 42:1101-1114.

Interagency Regulatory Liason Group. 1979. Scientific basis for identification of potential carcinogens and estimation of risks - Report of the work group of risk assessment. Journal Natl. Cancer Inst. 63 (1), July.

International Agency for Research in Cancer (IARC). 1977. The evaluation of the carcinogenic risk of chemicals to man. Monograph 15:111-138.

Johnson, J.E. 1971. The public health implications of widespread use of phenoxy herbicides and picloram. Bioscience 21:899-905.

Khera, K.S. and W.P. Mckinley. 1972. Pre- and postnatal studies on 2,4,5-trichlorophenoxyacetic acid, 2,4-dichlorophenoxyacetic acid and their derivatives in rats. Toxicol. Appl. Pharmacol. 22:14-28.

King, C.C. 1969. Effect of feeding herbicides to honey bees. North Central Weed Control Conference 17th p. 105.

Klingman, D.L., C.H. Gordon, G. Yip and H.P. Burchfield. 1966. Residues in the forage and in milk from cows grazing forage treated with esters of 2,4-D. Weeds 14:164-167.

Konstantinova, T.K. 1970. Toxicology and some problems of the embryotropic action of the butyl ester of 2,4-D. In: Vygodchikov, G.V. ed., Proceedings of a Conference on hygiene and toxicology of pesticides, USSR, 1967. Moscow, Meditsina. pp. 177-179.

Korte, C. and S.M. Jalal. 1979. 2,4-D induced alterations in mammalian chromosomes - preliminary results. North Dakota Academy of Science Proceedings 33:6.

Leng, M.L. 1972. Residues in milk and meat and safety of livestock from the use of phenoxy herbicides in pasture and range land. Down to Earth 28:12-20.

Loktionov, V.N., W.A. Budarkov, A.I. Kiznetsov and N.V. Kuznetsova. 1973. Toxicological characteristics of the manufacture of dichlorophenoxyacetic acid. Veterinariya (Moscow) 5:107-109.

Loos, M.A. 1975. In: Herbicides - Chemistry, Degradation and Mode of Action. P.C. Kearney and D.D. Kaufman (eds). Chapter 1: Phenoxyalkanoic Acids. Marcel Dekker, Inc., New York. p. 1-101.

McLaughlin, R.M. 1951. Toxicity of herbicides. Proc. Northeast Weed Control Control Conference 5:3-4.

Minnesota Department of Health (MDH). 1978. Assessment of human health risk associated with the use of 2,4-D in forestry management. Minneapolis, MN.

Moffett, J.O. and H.L. Morton. 1975. How herbicides affect honey bees. Am. Bee J. 115:178-179, 200.

Morton, H.L., E.D. Robinson and R.E. Meyer. 1967. Persistence of 2,4-D, 2,4,5-T and dicamba in range forage grasses. Weeds 15:268-271.

Morton, H.L., J.O. Moffett and R.H. MacDonald. 1972. Toxicity of herbicides to newly emerged honey bees. Environ. Entomol. 1:102-104.

Mullison, W.R. 1970. The significance of herbicides to non-target organisms. Proceedings of the Northeastern Weed Control Conference 24:111-147.

National Industrial Occupational Safety and Health (NIOSHA). 1978. Registry of Toxic Effects of Chemical Substances. p. 21,33,439.

Nielson, K., B. Kaempe and J. Jenson-Holm. 1965. Fatal poisoning in man by 2,4-dichlorophenoxyacetic acid (2,4-D). Determination of the agent in forensic materials. Acta. Pharmacol. Toxicol. 22:224-234.

Norris, L.A. 1966. Degradation of 2,4-D and 2,4,5-T in forest litter. J. For. 64:475-476.

Norris, L.A. 1967. Chemical brush control and herbicide residues in the forest environment. In: Symposium proceedings on herbicides and vegetation management in forests, range and noncrop lands. Oregon State Univ., Corvallis, OR. p.103-123.

Palmer-Jones, T. 1964. Effects on honeybees of 2,4-D. New Zealand J. of Ag. Research. 7:339-342.

Paulson, G.D. 1975. Fates of herbicides in animals. Residue Review 58:1-112.

Pilinskaya, M.A. 1974. Cytogenic effect of the herbicide 2,4-D on human and animal chromosomes. Tsitol. Genet. 8:202-206. (In Russian).

Prescott, L.F. 1969. British J. Clinical Pharmacol. 7:111-116.

Radionov, A.D., A.N. Chumachenko, and I.I. Kirilenko. 1967. The toxic properties of the herbicide 2,4-D. Hygiene and Sanitation 32:116-118.

Robertson, M.M. and R.C. Kirkwood. 1969. The mode of action of foliage-applied translocated herbicides with particular reference to the phenoxy acid compounds. II. The mechanism and factors influencing translocation, metabolism and biochemical inhibition. Weed Res. 10:94-120.

Rowe, V.K. and T.A. Hymas. 1954. Summary of toxicological information on 2,4-D and 2,4,5-T type herbicides and an evaluation of the hazards to livestock associated with their use. Am. J. Vet. Res. 15:622-629.

Sauerhoff, M.W., W.H. Braun, G.E. Blau and J.E. LeBeau. 1976. The fate of 2,4-dichlorophenoxyacetic acid (2,4-D) following oral administration to man. Toxicol. Appl. Pharmacol. 37:136-137.

Schillinger, J.I. 1960. (In German) J. Hyg. Epidemiol. (Praha). 4:243-252.

Schwetz, B.A., G.L. Sparschu and P.J. Gehring. 1971. The effects of 2,4-dichlorophenoxyacetic acid (2,4-D) and esters of 2,4-D on rat embryonal, foetal and neonatal growth and development. Food Cosmet. Toxicol. 9:801-817.

Seiler, J.P. 1978. The genetic toxicology of phenoxy acids other than 2,4,5-T. Mutation Research 55:226.

Stanley, C.W., J.E. Barney, W.R. Helton and A.R. Yobs 1971. Measurement of atmospheric levels of pesticides. Environ. Sci. Technol. 5:430-435.

Sund, J.M., M.J. Wright and J. Simon. 1960. Nitrates in weeds cause abortion in cattle. North Central Weed Control Conference Proceedings 13:31.

Torstensson, N.T.L., J. Stark and B. Goransson. 1975. The effect of repeated applications of 2,4-D and MCPA on their breakdown in soil. Weed Res. 15:159-164.

Torstensson, N.T.L. 1978. Effects of phenoxyacetic acid herbicides on soil. In: Chlorinated phenoxy acids and their dioxins. Ecological Bull. 27:263-284.

United States Enbironmental Protection Agency. 1980. Prehearing brief on the risks associated with the registered uses of 2,4,5-T and Silvex. 106pp.

United States Federal Regulation Code, Title 40, 1976. Protection of the Environment, part 180. Tolerances for pesticides in or on raw agricultural commodities. Office of the Federal Register, National Archives and Records Service. p. 61-63.

Waldron, A.C. 1974. Pesticide movement from cropland into Lake Erie. Ohio State University. Prepared for EPA Office of Water Resources Research. PB-235 650/9.

Warnock, J.W. and J. Lewis. 1978. The other face of 2,4-D - a citizens' report. South Okanagan Environmental Coalition, Penticton, British Columbia. 150pp.

Way, J.M. 1969. Toxicity and hazards to man, domestic animals and wildlife from some commonly used auxin herbicides. Residue Rev. 26-62.

Weed Science Society of America. 1974. Herbicide Handbook. p.43.

Wiese, A.F. and R.G. Davis. 1964. Herbicide movement in soil with various amounts of water. Weed Science 12:101-103.

Wojtalik, T.A., T.F. Hall and L.O. Hill. 1971. Monitoring ecological conditions associated with wide-scale applications of DMA 2,4-D to aquatic environments. Pestic. Monit. J. 4:184-203.

Yoder, J.M., M. Watson and W.W. Benson. 1973. Lymphocyte chromosome analysis of agricultural workers during extensive occupational exposure to pesticides. Mutation Research 21:335-340.

Young, A.L. et al., (not listed). 1978. The toxicology, environmental fate, and human risk of Herbicide Orange and it's associated dioxin.

Zetterberg, G. 1978. Genetic effects of phenoxy acids on microorganisma. In: Chlorinated phenoxy acids and their dioxins. Ecological Bull. 27:193-204.